CONFOCAL MICRO-FLOW VISUALIZATION OF BLOOD CELLS

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Abstract. *Progress in the development of confocal microscopy and the advantages of this technique over conventional microscopy have led to a new technique known as confocal micro-PTV. This technique combines a manual tracking method with a spinning disk confocal microscope. By combining its spatial filtering technique with a multipoint illumination system, this technique has the ability to obtain in-focus images with optical thickness less than 5 μm. The present study shows the ability of our confocal micro-PTV system to obtain detailed qualitative and quantitative information on the blood flow behavior in both glass capillaries and polydimethylsiloxane(PDMS) microchannels. By labeling the blood cells with a lipophilic carbocyanime derivative it was possible to measure both translational and rotational motion occurring during flow. Our results demonstrate the ability of our confocal micro-PTV system to obtain both translational and rotational motion of individual RBCs flowing in concentrated suspensions. Owing to its optical sectioning ability and consequent improvement of the image contrast and definition, the proposed confocal system can provide additional detailed description on the blood cells motion not obtainable by other conventional methods.*

1 INTRODUCTION

Phenomena of blood flow in microcirculation depend on several combined effects such as cell deformability, flow shear rates, vessel wall together with microscale biochemical and physiological factors. Since the availability of microscopic technique, extensive research on flow properties of blood was performed in both diluted and concentrated suspension of blood cells by using several measuring techniques such as double-slit photometry, video microscopy and image analysis, laser-Doppler anemometry, and particle-measuring methods [1]. Although the studies on the microhemodynamic behaviour of single red blood cell (RBC) in diluted suspensions are reaching to a mature state, its flow behaviour in highly concentrated suspensions is still not completely understood, partly due to technical limitations. Recently, considerable progress in the development of confocal microscopy and the advantages of this technique over conventional microscopy have led to a new technique known as confocal micro-PTV [1, 2]. This technique combines a manual tracking method with a spinning disk confocal microscope. By combining its outstanding spatial filtering technique with a multipoint illumination system, this technique has the ability to obtain in-focus images with optical thickness less than 5 μm. The present study shows the ability of our confocal micro-PTV system to obtain detailed qualitative and quantitative information on the blood flow behavior in both glass capillaries and polydimethylsiloxane (PDMS) microchannels.

2 MATERIALS & METHODS

2.1 Working fluids and RBCs labeling

The present study examined several working fluids: dextran 40 (Dx-40, Otsuka Medicine) containing 3%±1 (3Hct), 13%±1 (13Hct), 15%±1 (15Hct), 23%±1 (23Hct), 24%±1 (24Hct), 35%±1 (35Hct), and 37%±1 (37Hct) of human RBCs. The hematocrits (Hcts) correspond to the feed reservoir Hct and it was measured by using a hematocrit centrifuge (Kubota 3220, Japan).

The RBCs were fluorescently labeled with a lipophilic carbocyanime derivative dye, chloromethylbenzamido (CM-Dil, C-7000, Molecular Probes) using a procedure previously described elsewhere [1, 2].

2.2 Microchannels

In this study, we show results obtained in a 100 μm circular borosilicate glass microchannel fabricated by Vitrocom (Mountain Lakes, NJ, USA). The capillary was mounted on a slide glass with a thickness of 80±20 μm and was immersed in glycerol in order to minimize the refraction from the walls. Additionally, results from a 75 μm circular PDMS microchannels fabricated by a wire casting technique [3] were also analysed.

2.3 Confocal micro-PTV system

The confocal micro-PTV system used in this study consists of an inverted microscope (IX71; Olympus, Japan) combined with a confocal scanning unit (CSU22; Yokogawa, Japan), a diode-pumped solid-state (DPSS) laser (Laser Quantum, UK) with an excitation wavelength of 532 nm and a high-speed camera (Phantom v7.1; Vision Research, USA). The microchanels were placed on the stage of the inverted microscope

and by using a syringe pump (KD Scientific, USA) a pressure-driven flow was kept constant (Re from ~ 0.005 to ~ 0.007). Moreover, by using a thermo plate controller (Tokai Hit) the temperature surrounding the microchannels was 36ºC±1.

The laser beam was illuminated from below the microscope stage and the light emitted from the fluorescent flowing RBCs passes through a color filter into the scanning unit CSU22, where by means of a dichromatic mirror, is reflected onto a high speed camera to record the confocal images. The middle plane images were captured, digitized and transferred to a computer for evaluation using a Phantom camera control software (PH607). A manual tracking plugin (MTrackJ) [4] of an image analysis software (Image J, NIH) [5] was used to track the labeled RBCs. By using this software, the bright centroid of the selected RBC was automatically computed through successive images so that it was possible to track accurately the labeled RBCs even when two cells were close together, which is the case of cell-cell interaction. A more detailed description of the confocal micro-PTV/PIV system used in this study can be found elsewhere [1, 2, 6, 7].

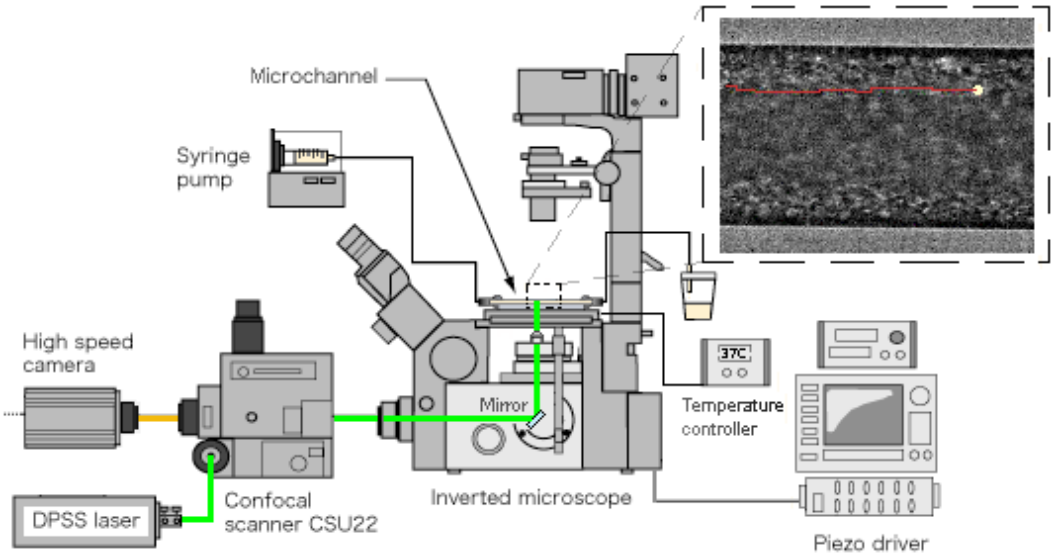

Figure 1: Schematic diagram of the confocal micro-PTV system.

2.4 RBC radial dispersion coefficient

The detailed measurements on the motion of individual RBCs at a microscopic level are crucial to elucidate both mesoscopic and macroscopic blood flow properties. One accepted way to correlate the microscopic events to the macroscopic flow behaviour is by calculating the radial dispersion coefficient (D_{yy}) [8]. In the present study, the RBC radial dispersion coefficient (D_{yy}) is given by :

$$D_{yy} = \sum_{t=0}^{n} \frac{\left\langle \left(R_y(t) - R_y(0)\right)^2 \right\rangle}{2t} \qquad (1)$$

where R_y and t are the radial displacement and time interval respectively.

3 RESULTS & DISCUSSION

To analyse the ability of the confocal micro-PTV system to track RBCs, the motions of labeled RBCs were followed at several Hcts (3% to 37%). For the calculation of the radial dispersion coefficient (D_{yy}), measurements were performed at middle plane of the microchannels. However to investigate complex microrhelogical events in flowing blood (such as interaction and orientation of blood cells) all measurements were performed near the wall of the microchannel (z = 20 μm) with Hct ~ 20% and Re ~ 0.007.

3.1 RBC-RBC interactions in flowing blood

The effect of the hemodynamic interactions on the motion of RBCs depend on multi-physic factors, such as shear rate, deformability, plasma layer and wall constriction. Figure 2 shows the trajectories of two-RBC interactions near the wall and within the plasma layer. This figure shows the radial disturbance effect enhanced by the collision of a neighboring RBC.

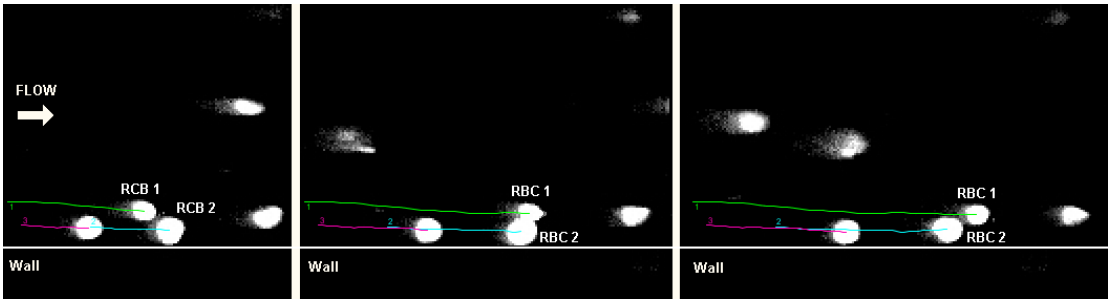

Figure 2: Two-RBC interactions at different time intervals.

3.2 RBC-WBC interactions in flowing blood

The hemodynamic interaction effect of WBC on the motion of RBCs was also measured. Figure 3 shows a RBC interacting with centre upper part of a WBC. It is possible to observe that the transversal displacement increases when the collision with a WBC occurred.

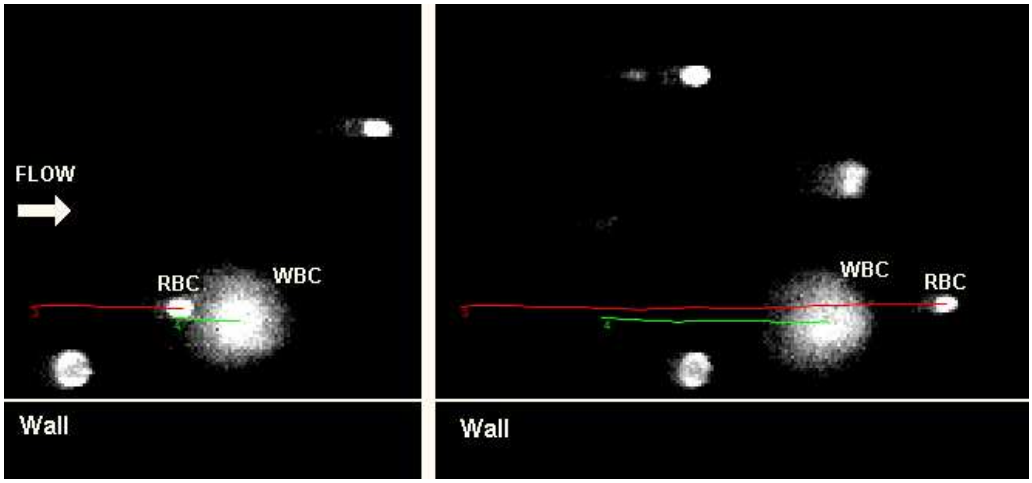

Figure 3: RBC-WBC interaction at different time intervals (adapted from [2]).

3.3 Orientation of RBC in flowing blood

Although the orientation of RBCs was extensively studied at low Hct (Hct < 1%), such is not the case for moderate and high Hcts. Figure 4 shows the orientation of a RBC flowing nearby the wall of the glass microchannel. By adjusting the image contrast, it was possible to measure both translational and rotational motion. The translational motion was measured at the centre of the RBC whereas the rotational was measured along the membrane as shown in Figure 4.

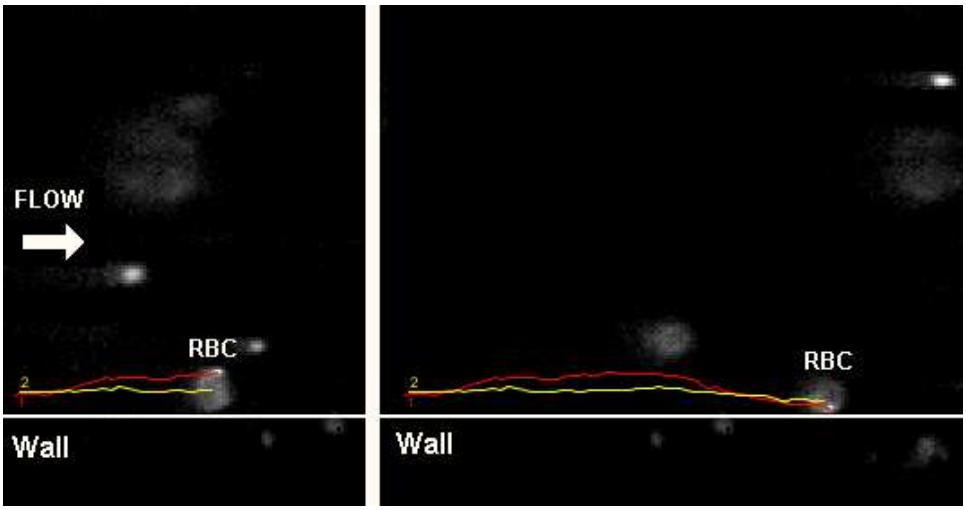

Figure 4: Translational and rotational motion of a RBC.

3.4 Effect of Hct on the RBC trajectories

Figure 5 shows the trajectory of a RBC with any appreciable interaction at 3% Hct. Additionally, it also shows the trajectory of a flowing RCB suffering multiple interactions from neighboring RBCs at 20% Hct. This latter RBC path exhibit higher erratic displacements in the direction normal to the flow. These qualitative results show clearly the fluid-dynamical interaction effect on the motion of RBCs flowing in concentrated suspension of blood cells (20% Hct). Hence, these results shows evidence that RBCs at dense concentrations exhibit higher erratic transversal displacement when compared with diluted suspensions of RBCs.

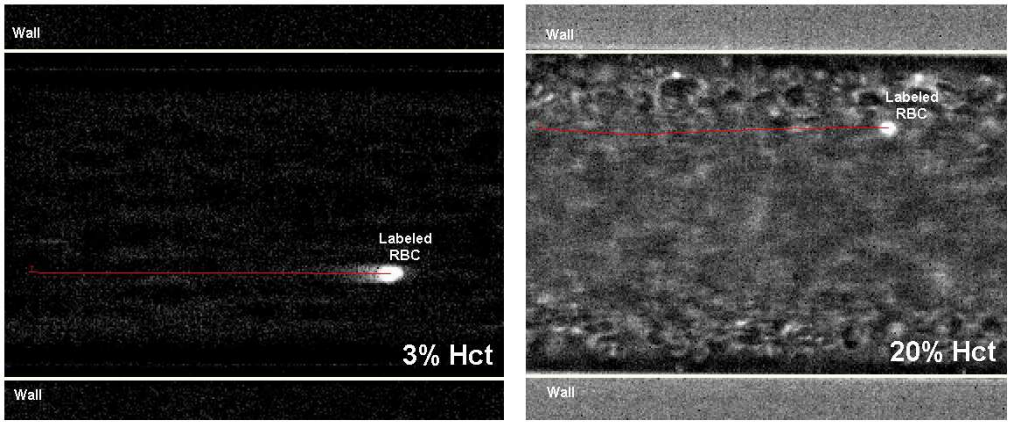

Figure 5: Comparison of the trajectories between a RBC with no interactions at low Hct (3%Hct) and a RBC with multiple interactions (20% Hct) (adapted from [9]).

3.5 Radial dispersion of labeled RBCs at different Hcts

For the present study, in vitro blood with several Hct was used at Re ~ 0.005. The paths of hundreds labeled RBCs were measured in the centre plane of both glass and PDMS circular microchannel. The RBC dispersion coefficient (D_{yy}) for two different diameters (75μm and 100μm) and for several Hcts is shown in Figure 6. The results show evidence not only that D_{yy} rises with the increase of the Hct but also that D_{yy} tends to decrease with the diameter.

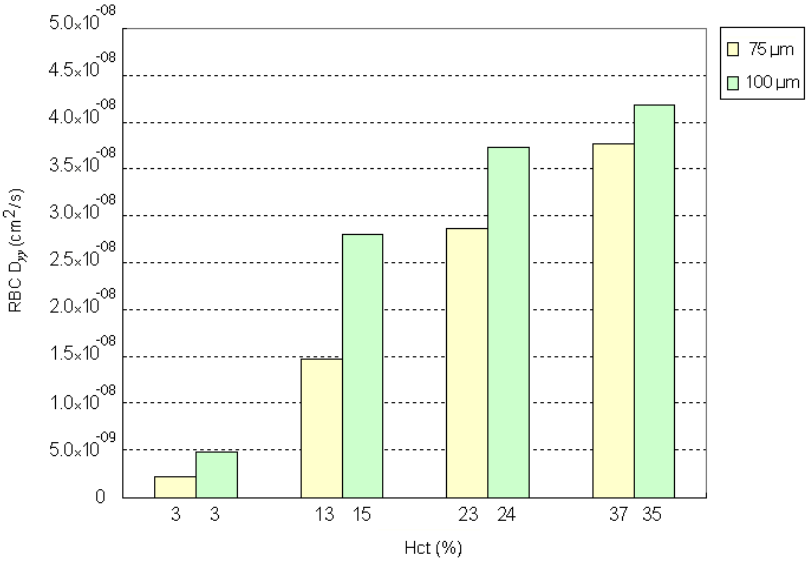

Figure 6: Effect of the Hct on the RBC D_{yy} at 75μm PDMS microchannel and 100μm glass capillary (adapted from [3, 9]).

4 CONCLUSION

The present study shows the ability of our confocal micro-PTV system to obtain detailed qualitative and quantitative information on the blood flow behaviour in both glass capillaries and polydimethylsiloxane(PDMS) microchannels. By labeling the blood cells with a lipophilic carbocyanime derivative, it was possible to measure translational and rotational motion, occurring during flow, in both diluted and concentrated suspensions of RBCs.

The experiments were performed in the middle plane of 100μm glass microtube and 75μm circular PDMS microchannel at low Reynolds numbers (Re from ~ 0.005 to ~ 0.007) and by using Hcts from 3% up to 37%. The results suggest that the RBC paths are strongly dependent on the Hct and as a result the RBC dispersion coefficient increases with the Hct.

Owing to its optical sectioning ability and consequent improvement of the image contrast and definition, the proposed confocal system work is proved to be a powerful technique to provide additional detailed description on the blood cells motion not obtainable by other conventional methods.

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